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(54) LIVRAISON LOCALE DE 17-B ESTRADIOL POUR LA PREVENTION DE L'ANGIOPLASTIE TRANSLUMINALE PERCUTANEE

(54) LOCAL DELIVERY OF 17 - BETA ESTRADIOL DURING BALLOON ANGIOPLASTY FOR PREVENTING RESTENOSIS

(57) The endothelium plays a major role in the regulation of structural and functional integrity of coronary arteries. Damage to endothelium occurs following percutaneous transluminal coronary angioplasty (PTCA). Locally delivered 17 - beta estradiol following PTCA in pigs resulted in an enhanced reendothelialization and endothelial function, possibly via a markedly higher endothelial nitric oxide synthase (eNOS) expression when compared to controls. Since endothelial dysfunction can promote both restenosis and coronary spasm, local 17 - beta estradiol delivery is a promising new approach to improve the results after PTCA.

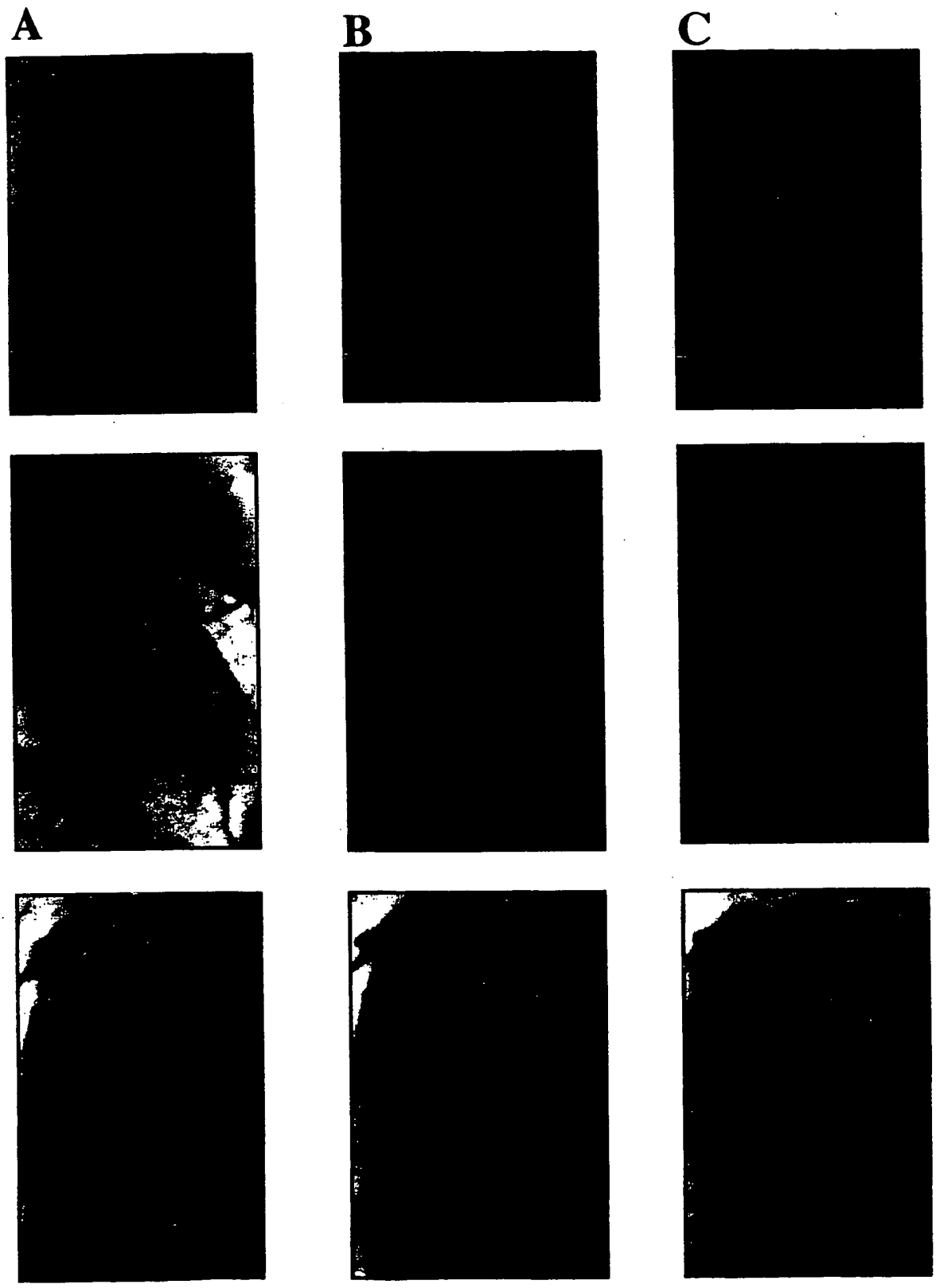


FIGURE 1

A

B

C

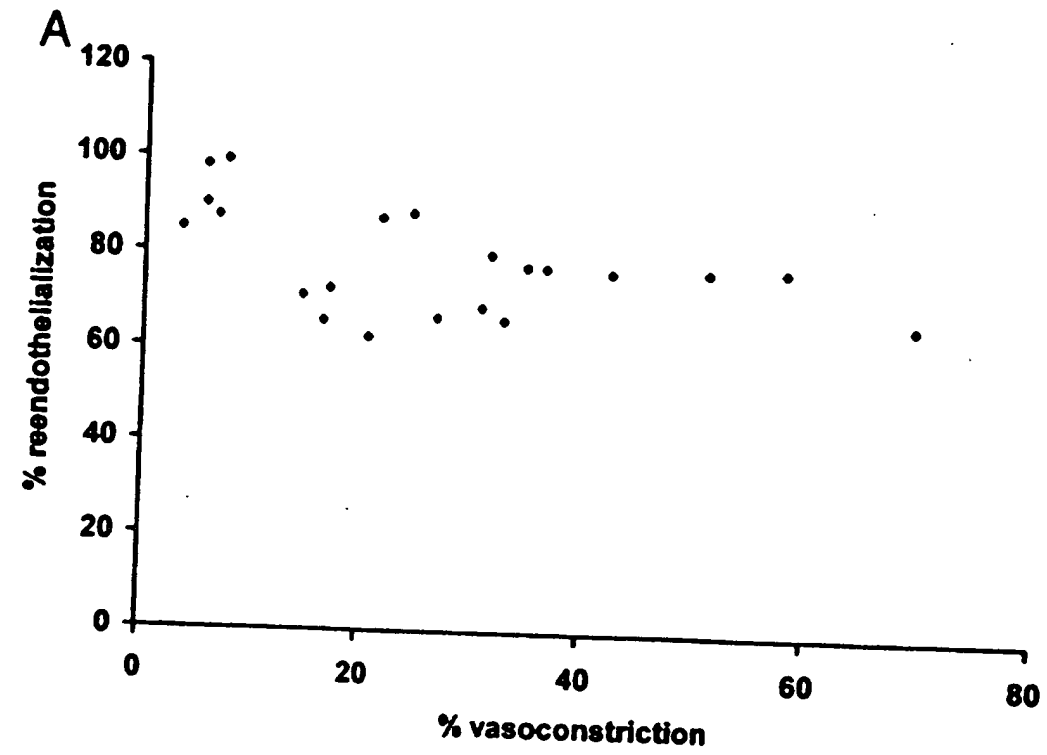
FIGURE 2

A

B

C

FIGURE 3


A
120
100
80
60
40
20
0
% reendothelialization
0
20
40
60
80
% vasoconstriction

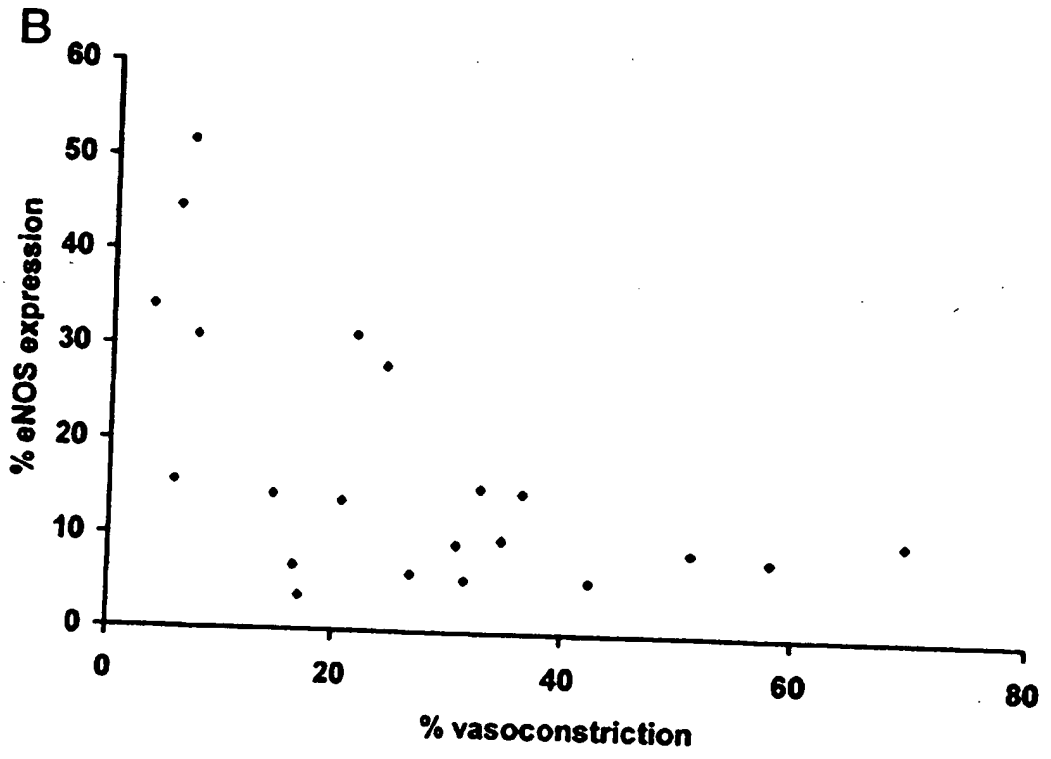

B
60
50
40
30
20
10
0
% eNOS expression
0
20
40
60
80
% vasoconstriction

FIGURE 4

TITLE OF THE INVENTION

Local Delivery of 17 - beta Estradiol During Balloon Angioplasty for Preventing Restenosis.

BACKGROUND OF THE INVENTION

The vital role of endothelium in the regulation of vascular tone of arteries is well recognized (1). The intact endothelium also has important inhibitory effects on platelet aggregation, monocyte adhesion, and vascular smooth muscle cell proliferation (2). Endothelial injury associated with endothelial dysfunction is known to occur as a consequence of percutaneous transluminal coronary angioplasty (PTCA) (3), and may play an important role in restenosis following PTCA (4). Impaired endothelial function has been demonstrated in porcine coronary arteries as long as 4 weeks following PTCA in pigs (5). Systemically administered 17 - beta estradiol has been reported to accelerate endothelial recovery after arterial injury (6). Since endothelial injury due to PTCA is a local event, we hypothesized that local delivery of 17 - beta estradiol following PTCA may enhance endothelial recovery.

DESCRIPTION OF INVENTION

Animal preparation

The study protocol was approved by the Animal Care and Ethical Research Committee of the Montreal Heart Institute. Juvenile farm pigs weighing 20-25 kg (1 female, and 8 castrated males) were used. On the day of the experiment, animals received 650 mg of acetylsalicylic acid and 30 mg of nifedipine orally, were premedicated with 6mg/kg of tiletamine hydrochloride and zolazepam hydrochloride, and were given 0.05 mg of atropine intramuscularly. Under general anesthesia (a mixture of 1-1.5% isoflurane and oxygen enriched air), the right femoral artery was cannulated percutaneously. An 8 Fr arterial sheath was introduced, and 100 mg/kg of lidocaine and 250 U/kg of heparin were

administered intra-arterially. Additional heparin was administered during PTCA if needed, to maintain an activated coagulation time of > 300 seconds.

Procedure

An 8 Fr right Amplatz guiding catheter and right Judkins guiding catheter were used for cannulation of the left and right coronary arteries, respectively. A standard balloon catheter (corresponding to a balloon/artery ratio of 1.1-1.3:1) was advanced over a 0.014" floppy guide wire, and 3 successive 30-second inflations at 10 atm pressure were made with a 30 second interval between each inflation. PTCA was performed on all 3 coronary arteries of each animal. For local delivery, the InfusaSleeve catheter (LocalMed Inc.) was used, which permits safe drug delivery with negligible additional injury (7). After balloon dilatation, each coronary artery of an animal was randomized to receive either 600 µg of 17 – beta estradiol (in 5 ml), vehicle alone (5 ml), or PTCA only. The vehicle 2-hydroxypropyl-beta-cyclodextrin (HPCD), and 17 – beta estradiol were obtained from Sigma Chemical Co. For local delivery with the InfusaSleeve catheter, a proximal driving pressure of 10 atm and support balloon pressure of 6 atm were utilized.

Intracoronary infusion

All 9 animals underwent cardiac catheterization at the end of 4 weeks. After a baseline coronary angiogram, selective cannulation of the proximal portion of a coronary artery was performed with a single lumen balloon catheter (TotalCross, Schneider) for the administration of vasoactive agents. Acetylcholine (Ach) in increasing concentrations of

10^{-7} M, 10^{-6} M, 10^{-5} M, and 10^{-4} M was successively infused through the lumen port of the catheter. Each dose was administered for a duration of 3 minutes at a constant rate of 1 ml/min using an infusion pump. Coronary angiography was performed at the end of each dose. After infusion of the highest concentration of Ach (10^{-4} M) and angiography, 100 µg of nitroglycerin was administered via the lumen port of the catheter, and a coronary angiogram performed. The same protocol was repeated for the other 2 coronary arteries. Heart rate, blood pressure, and ECG were monitored continuously throughout the experiment.

Quantitative coronary angiography

Coronary angiography was performed with a single plane imaging system (Electromed Intl). Images were obtained in predetermined views which best demonstrated the vessel segment of interest, and without overlap of branches. Care was taken to maintain the same angulation during angiography of a segment throughout the procedure. Ionic contrast (MD-76, Mallinckrodt Medical Inc) was used throughout the experiment. Images were captured at a frame speed of 30 frames/sec, and stored digitally. A segment of contrast-filled guiding catheter was included in every frame, for the purpose of calibration. Calibration was performed using the known diameter of the contrast-filled guiding catheter as the reference segment, to avoid error due to magnification. Coronary artery diameter measurements were made using a validated computerized edge-detection system (8). The midpoint of the injured segment was used for calculation of coronary artery diameter. For each analysis, coronary artery diameter measurements were

performed in 3 consecutive end-diastolic frames, and the results averaged. Measurements were performed by an independent observer blinded to the treatment group of the vessels.

Immunohistochemistry

The animals were euthanized at 4 weeks. Under general anaesthesia as described above, exsanguination was performed with replacement by 1 l of 0.9 % NaCl solution. The heart was perfusion-fixed in vivo with 2 l of 10 % buffered formalin at 200 mm Hg pressure. The heart was then removed, and the coronary arteries were harvested immediately. From the injured segment (identified in relation to side branches), serial sections of 3-5 mm were made, and stored in 10 % buffered formalin solution. The sections were then treated with incremental concentrations of alcohol, followed by treatment with xylene and paraffin. Slices of 6 µm thickness were prepared, and stained with Verhoeff's stain for assessment of tissue response to injury. For each injured segment, 2 slices demonstrating maximal neointimal response were selected for immunohistochemistry, and the results obtained from analysis of the cross sections were averaged. The % of reendothelialization and, the % of endothelial nitric oxide synthase (eNOS) expression were calculated as follows: (the total length of the luminal surface staining positively / the perimeter of the lumen) x 100, respectively. Analysis was performed by an independent examiner with no knowledge of the treatment groups to which the sections belonged. For lectin immunohistochemistry, the 6 µm slices were first treated with hydrogen peroxide and methan l to block endogenous peroxide, incubated with the *Dolichos biflorus* agglutinin (Sigma Chemical Co.) followed by treatment with 3,3'-diaminobenzidine (Vector Laboratories) and, subsequently counter-stained with hematoxylin. For immunohist chemistry of eNOS expression, after blocking

f endogenous peroxide and non-specific antibodies, the slices were treated serially with the primary mouse anti-eNOS antibody (Bio/Can Scientific), the secondary goat anti-mouse antibody (Vector Laboratories), incubated with avidin-biotin (Vector Laboratories), treated with 3,3'-diaminobenzidine (Vector Laboratories) and finally counter-stained with hematoxylin. For both immunohistochemical examinations, normal porcine carotid artery slices were used as positive controls; whereas slices obtained from the injured coronary arteries and stained only with hematoxylin were used as negative controls.

Statistical analysis

Values are expressed as mean ± SD. Comparison of basal coronary artery diameter among the 3 groups was made using the one-way analysis of variance test. Comparisons between basal coronary artery diameter and coronary artery diameter following infusion of vasoactive agents were made with two-tailed Student's t-tests. The Kruskal-Wallis test was used for comparison of lectin and eNOS expression among the 3 treatment groups. Linear relationships between lectin expression and response to Ach, and between eNOS expression and response to Ach were analyzed with Pearson correlation coefficients. Values were considered to be statistically significant if $p < 0.05$.

Results

There were no significant differences in basal coronary artery diameter (2.53 ± 0.6 mm for 17 – beta estradiol, 2.79 ± 0.35 mm for PTCA only, and 2.77 ± 0.44 mm for vehicle

groups respectively, p = 0.4) among the 3 treatment groups. The extent of morphologic tissue injury (9) among the groups was similar. No changes in heart rate, ECG, or blood pressure were noted during the local delivery or during intracoronary infusion of vasoactive agents.

Response of PTCA only group to Ach

Compared to the basal coronary artery diameter, there were no significant changes in coronary artery diameter following intracoronary infusion of 10^{-7} M, and 10^{-6} M concentrations of Ach (Table). At a concentration of 10^{-5} M, a significant vasoconstrictive response was noted ($p < 0.02$). A marked vasoconstrictive response was observed at a concentration of 10^{-4} M ($p < 0.0001$) (Figure 1). The vasoconstriction was completely reversed upon administration of the endothelium-independent vasodilator nitroglycerin. Coronary diameter increased from 1.8 ± 0.48 mm after 10^{-4} M Ach, to 2.5 ± 0.28 mm following nitroglycerin ($p < 0.01$; $p = 0.2$ for post-nitroglycerin vs basal diameter).

Response of vehicle treatment group to Ach

Compared to the basal coronary artery diameter, 10^{-7} M Ach did not change coronary artery diameter in the vehicle treatment group (Table). A trend towards significant vasoconstriction was noted with 10^{-6} M Ach ($p = 0.06$). Significant vasoconstriction was produced by 10^{-5} M ($p < 0.02$), and at 10^{-4} M ($p < 0.001$) Ach infusion respectively (Figure 1). Nitroglycerin completely reversed the vasoconstriction, returning the arteries

to their basal diameter (from 1.89 ± 0.51 mm after 10^{-4} M Ach, to 2.69 ± 0.52 mm following nitroglycerin [$p < 0.004$; $p = 0.7$ for post-nitroglycerin vs basal diameter]).

Response of 17 – beta estradiol treated group to Ach

In the vessels treated with local delivery of 17 – beta estradiol, no significant vasoconstrictive response to Ach occurred at any concentration used (Table) (Figure 1). A mild and statistically nonsignificant increase in coronary artery diameter was observed following administration of nitroglycerin: from 2.28 ± 0.61 mm after 10^{-4} M Ach to 2.61 ± 0.48 mm after nitroglycerin ($p = 0.4$; $p = 0.8$ for post-nitroglycerin vs basal diameter).

Immunohistochemistry

Immunohistochemical analyses were performed 4 weeks after PTCA on all 9 animals. Three arterial segments were lost/damaged during harvesting of the samples (2 of PTCA only group, and 1 of vehicle group). Significant differences were seen among the 3 treatment groups in the extent of re-endothelialization, as assessed by immunohistochemical analysis with the lectin *Dolichos biflorus* agglutinin (Figure 2). Re-endothelialization was noted to a greater extent in vessels treated with local delivery of 17 – beta estradiol, compared to the other 2 groups (90.6 ± 5.5 % for 17 – beta estradiol, 71 ± 6.8 % for PTCA only, and 72.8 ± 4.9 % for vehicle, $p < 0.0005$). Endothelial nitric oxide synthase expression was also higher in vessels treated with 17 – beta estradiol (35.6 ± 11.8 % for 17 – beta estradiol, 9.4 ± 3.9 % for PTCA only, and 9.2

± 4.0 % for vehicle, $p < 0.0005$) (Figure 3). No significant differences in immunohistochemical analyses were observed between vessels treated with vehicle or PTCA only.

We proceeded further to analyze whether a linear relationship between reendothelialization and the response to Ach could be demonstrated. A significant inverse correlation was noted between reendothelialization as assessed by immunohistochemistry with the lectin *Dolichos biflorus* agglutinin and the response to Ach ($r = - 0.48$, $p < 0.02$) (Figure 4). An even stronger inverse linear correlation was observed between eNOS expression and the response to Ach ($r = -0.58$, $p < 0.005$).

Discussion

This study demonstrates for the first time that local delivery of 17 – beta estradiol immediately following PTCA enhances subsequent reendothelialization and endothelial function at the site of injury. Besides its critical role in the regulation of vascular tone, the normal endothelium functions as an effective barrier between blood elements and underlying vascular smooth muscle cells. Endothelium-derived nitric oxide (NO) is a potent vasodilator, inhibits monocyte adherence and platelet aggregation and adhesion (10), vascular smooth muscle cell migration (11) and proliferation (12).

PTCA is associated with arterial injury and damage to the endothelium (3). Following arterial injury, varying rates of reend thelialization have been reported. Reendothelialization rates of 81 % (13), and even lower rates of < 50 % (14) following

arterial injury have been observed. In a study of specimens of restenotic lesions obtained by atherectomy in humans, no endothelial cells could be demonstrated (15). In the present study, local treatment with 17 – beta estradiol was followed by nearly complete reendothelialization (90.6 ± 5.5 %), which was significantly greater than that observed in the groups not treated with 17 – beta estradiol. Estrogen receptors have been identified in human coronary artery and umbilical vein endothelial cells (16), and when bound to estrogen are capable of regulating protein synthesis by altering transcription rates (17). In cell culture assay of human umbilical vein endothelial cells, treatment with 17 – beta estradiol markedly increased both cell migration and proliferation (18). Therapy with subcutaneously implanted 17 – beta estradiol pellets significantly enhanced reendothelialization following arterial injury (6). The capacity of 17 – beta estradiol to increase vascular endothelial growth factor synthesis (19) and the effect of 17 – beta estradiol on basic fibroblast growth factor may be responsible for the enhanced reendothelialization. Vascular endothelial growth factor treatment is known to promote reendothelialization in vivo (20). In human umbilical vein and coronary artery endothelial cell culture experiments, treatment with 17 – beta estradiol enhanced the release and phosphorylation of basic fibroblast growth factor (21,22). It has been shown that administration of basic fibroblast growth factor in vivo stimulates reendothelialization following arterial injury in rats (23). Another mechanism by which 17 – beta estradiol could possibly influence extent of reendothelialization is by inhibition of apoptosis of injured endothelial cells: a 50 % decrease in apoptosis was seen with 17 – beta estradiol treatment of human umbilical vein endothelial cells exposed to tumor necrosis factor-α

(24). It is noteworthy that increased expression of tumor necrosis factor-α is known to occur following balloon injury (25).

Impaired endothelial function, as in atherosclerosis (26) or following experimental inhibition of NO (27), has been associated with a paradoxical constrictive response to Ach. This paradoxical response to Ach could be modified by treatment with estrogen. In humans, 17 – beta estradiol, administered intravenously (28) or by continuous intracoronary infusion (29), attenuated the vasoconstrictive response to Ach and also inhibited the Ach-induced increase in coronary resistance and decrease in coronary blood flow. The regulatory effect of 17 – beta estradiol on eNOS that we observed may be responsible for the beneficial effects on endothelial function, as vascular response to Ach is closely related to eNOS expression (30,31). In support of this notion, a strong inverse linear relationship was seen between the vascular response to Ach and eNOS expression (Figure 4). The ability of estrogen to induce nitric oxide synthase was first identified during gestation in guinea pigs (32). Induction of eNOS function by 17 – beta estradiol has been subsequently demonstrated to be accompanied by increased eNOS protein and mRNA expression (33,34). Increased circulating NO levels have been observed in postmenopausal women treated with 17 – beta estradiol (35). Following arterial injury, the regenerated endothelium is often functionally abnormal (5). Abnormal vasomotion as a result of persistent endothelial dysfunction at the site of angioplasty has been demonstrated in patients undergoing PTCA, and is postulated to be responsible for the symptom of angina noted in patients with nonsignificant stenosis following PTCA (36). We have shown that functional abnormalities could be improved significantly by treatment with locally delivered 17 – beta estradiol. A unifying hypothesis for the

responses we observed is that eNOS downregulation following PTCA prevents the vasodilatory response to Ach mediated by endothelial NO production. By improving eNOS expression, 17 – beta estradiol allows the vasodilatory response of Ach to counteract its direct vasoconstricting action, preventing Ach-induced vasoconstriction at the site of local injury. The vasodilatory response to nitroglycerin in Ach-constricted arteries post-PTCA is consistent with this concept, since exogenous nitroglycerin (which is a NO donor) simply provides a local NO-related dilation that the eNOS deficient angioplastied segment cannot provide for itself.

Both rapid non-genomic and genomic effects have been postulated to be involved in the influence of 17 – beta estradiol on coronary vasculature (37,38). Although increased protein synthesis was not quantified in the present study, the enhanced eNOS expression and the response to Ach observed as late as 28 days following a single dose of 17 – beta estradiol appears to be consistent with a genomic effect. This is the first study to suggest the existence of a genomic effect following local therapy with 17 – beta estradiol in coronary circulation in vivo.

Gender differences in the endothelium-dependent vasodilation by 17 – beta estradiol have been noted (39). In our study, a majority of animals were males, and a significant beneficial effect of 17 – beta estradiol was noted in all the animals studied, irrespective of sex. Thus, local delivery of 17 – beta estradiol appears to be effective in males as well as females. There is evidence to suggest that the simultaneous administration of progesterone reduces NO levels induced by 17 – beta estradi l (35), this issue was, however, beyond the scope of the present study.

We conclude that a single dose of 17 - beta estradiol delivered locally following balloon injury can significantly improve reendothelialization and enhance endoth lial function at the injured site as late as 1 month following injury. Besides the beneficial vascular effects of improved endothelial function, this observation may be of particular importance following balloon angioplasty as improved endothelial function is known to be associated with decreased neointima formation in the injured area (20,40). This approach merits further study, with a view to potential clinical value in the prevention of vascular dysfunction and restenosis following PTCA.

Although the present invention has been described hereinabove by way of a preferred embodiment thereof, this embodiment can be modified at will, within the scope of the appended claims, without departing from the spirit and nature of the subject invention.

References

1. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373-6.
2. Rubanyi GM. The role of endothelium in cardiovascular homeostasis and diseases. J Cardiovasc Pharmacol 1993; 22(Suppl. 4): S1-S14.
3. Fischell TA, Derby G, Tse TM, Stadius ML. Coronary artery vasoconstriction routinely occurs after percutaneous transluminal coronary angioplasty: a quantitative arteriographic analysis. Circulation 1988; 78: 1323-34.
4. Chesebro JH, Lam JY, Badimon L, Fuster V. Restenosis after arterial angioplasty: a hemorrheologic response to injury. Am J Cardiol 1987; 60: 10B-16B.
5. Shimokawa H, Aarhus LL, Vanhoutte PM. Porcine coronary arteries with regenerated endothelium have a reduced endothelium-dependent responsiveness to aggregating platelets and serotonin. Circ Res 1987; 61: 256-70.
6. Krasinski K, Spyridopoulos I, Asahara T, et al. Estradiol accelerates functional endothelial recovery after arterial injury. Circulation 1997; 95: 1768-72.
7. Moura A, Lam JYT, Hebert D, et al. Intramural delivery of agent via a novel drug-delivery sleeve: histologic and functional examination. Circulation 1995; 92: 2299-2305.
8. Mancini GBJ, Simon SB, McGillem MJ, et al. Automated quantitative coronary arteriography: morphologic and functional validation in vivo of a rapid digital angiographic method. Circulation 1987; 75(2): 452-60.

9. Karas SP, Gravanis MB, Santoian EC,et al. Coronary intimal proliferation after balloon injury and stenting in swine: an animal model of restenosis. J Am Coll Cardiol 1992; 20: 467-74.

10. Cooke JP, Tsao PS. Cytoprotective effects of nitric oxide. Circulation 1993; 88(5): 2451-4.

11. Sarkar R, Meinberg EG, Stanley JC, et al. Nitric oxide reversibly inhibits the migration of cultured vascular smooth muscle cells. Circ Res 1996; 78: 225-230.

12. Cornwell TL, Arnold E, Boerth NJ, Lincoln TM. Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. Am J Physiol 1994; 267: C1405-13.

13. Hayashi Y, Tomoike H, Nagasawa K, et al. Functional and anatomical recovery of endothelium H1090.

14. Lindner V, Reidy MA, Fingerie J. Regrowth of arterial endothelium: denudation with minimal trauma leads to complete endothelial cell growth. Lab Invest 1989; 61: 556-63.

15. Bauriedel G, Windstetter U, DeMario Jr SJ, et al. Migratory activity of human smooth muscle cells cultivated from coronary and peripheral primary and restenotic lesions removed by percutaneous atherectomy. Circulation 1992; 85: 554-64.

16. Kim-Schulze S, McGowan KA, Hubchak SC, et al. Expression of an estrogen receptor by human coronary artery and umbilical vein endothelial cells. Circulation 1996; 94: 1402-7.

17. Venkov CD, Rankin AB, Vaughan DE. Identification of authentic estrogen receptor in cultured endothelial cells: a potential mechanism for steroid hormone regulation of endothelial function. Circulation 1996; 94: 727-33.

18. Morales DE, McGowan KA, Grant DS, et al. Estrogen promotes angiogenic activity in human umbilical vein endothelial cells in vitro and in a murine model. Circulation 1995; 91: 755-63.after denudation of coronary artery. Am J Physiol 1988; 254: H1081-

19. Hyder SM, Stancel GM, Chiappetta C, et al. Uterine expression of vascular endothelial growth factor is increased by estradiol and tamoxifen. Cancer Res 1996; 56(17): 3964-60.

20. Asahara T, Bauters C, Pastore C, et al. Local delivery of vascular endothelial growth factor accelerates reendothelialization and attenuates intimal hyperplasia in balloon-injured rat carotid artery. Circulation 1995; 91: 2793-2801.

21. Kim-Schulze S, Lowe WL, Schnapper HW. Estrogen stimulates delayed mitogen-activated protein kinase activity in human endothelial cells via an autocrine loop that involves basic fibroblast growth factor. Circulation 1998; 98: 413-21.

22. Albuquerque ML, Akiyama SK, Schnaper HW. Basic fibroblast growth factor release by human coronary artery endothelial cells is enhanced by matrix proteins, 17 – beta estradiol, and a PKC signaling pathway. Exp Cell Res 1998; 245(1): 163-9.

23. Lindner V, Majack RA, Reidy MA. Basic fibroblast growth factor stimulates endothelial regrowth and proliferation in denuded arteries. J Clin Invest 1990; 85: 2004-8.

24. Spyridopoulos I, Sullivan AB, Kearney M, et al. Estrogen-receptor – mediated inhibition of human endothelial cell apoptosis: estradiol as a survival factor. Circulation 1997; 95: 1505-14.

25. Tanaka H, Sukhova G, Schwartz D, Libby P. Proliferating arterial smooth muscle cells after balloon injury express TNF-α but not interleukin-1 or basic fibroblast

growth factor. Arterioscler Thromb Vasc Biol 1996; 16: 12-18.

26. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Eng J Med 1986; 315: 1046-51.

27. Collins P, Burman J, Chung H, Fox K. Hemoglobin inhibits endothelium-dependent relaxation to acetylcholine in human coronary arteries in vivo. Circulation 1993; 87: 80-5.

28. Reis SE, Gloth ST, Blumenthal RS, et al. Ethinyl estradiol acutely attenuates abnormal coronary vasomotor responses to acetylcholine in postmenopausal women. Circulation 1994; 89: 52-60.

29. Gilligan DM, Quyyumi AA, Cannon III RO. Effects of physiological levels of estrogen on coronary vasomotor function in postmenopausal women. Circulation 1994; 89: 2545-51.

30. Seo KK, Yun HY, Kim H, Kim SC. Involvement of endothelial nitric oxide synthase in the impaired endothelium-dependent relaxation of cavernous smooth muscle in hypercholesterolemic rabbit. J Androl 1999; 20(2): 298-306.

31. Kullo IJ, Mozes G, Schwartz RS, et al. Enhanced endothelium-dependent relaxations after gene transfer of recombinant endothelial nitric oxide synthase to rabbit carotid arteries. Hypertension 1997; 30(part 1): 314-20.

32. Weiner CP, Lizasoain I, Baylis SA, et al. Induction of calcium-dependent nitric oxide synthases by sex hormones. Proc Natl Acad Sci USA 1994; 91: 5212-16.

33. Hishikawa K, Nakaki T, Marumo T, et al. Up-regulation of nitric oxide synthase by estradiol in human aortic endothelial cells. FEBS Letters 1995; 360: 291-3.

34. MacRitchie AN, Jun SS, Chen Z, et al. Estrogen upregulates endothelial nitric oxide synthase gene expression in fetal pulmonary artery endothelium. Circ Res 1997; 81:

355-62.

35. Rosselli M, Imthurn B, Keller PJ, et al. Circulating nitric oxide (nitrite/nitrate) levels in postmenopausal women substituted with 17β-estradiol and norethisterone acetate: a two-year follow-up study. Hypertension 1995; 25(part 2): 848-53.

36. Malekianpour M, Doucet S, Lesperance J, et al. Abnormal coronary vasomotion and angina after successful coronary angioplasty. Circulation 1996; 94(suppl I): I-560.

37. Williams JK, Adams MR, Herrington DM, Clarkson TB. Short-term administration of estrogen and vascular responses of atherosclerotic coronary arteries. J Am Coll Cardiol 1992; 20: 452-7.

38. Wellman GC, Bonev AD, Nelson MT, Brayden JE. Gender differences in coronary artery diameter involve estrogen, nitric oxide, and Ca^{2+}-dependent K^+ channels. Circ Res 1996; 79: 1024-30.

39. Kawano H, Motoyama T, Kugiyama K, et al. Gender differences in improvement of endothelium-dependent vasodilation after estrogen supplementation. J Am Coll Cardiol 1997; 30: 914-9.

40. Chandrasekar B, Tanguay JF. Local delivery of 17 – beta estradiol decreases neointimal hyperplasia following coronary angioplasty in porcine model. (Submitted for publication).

Legends

Figure 1: Representative coronary angiograms demonstrating the vasoconstrictive response to intracoronary infusion of acetylcholine (Ach) 10^{-4} M, obtained from the same animal at 4 weeks following percutaneous transluminal coronary angioplasty (PTCA). Column A = basal , column B = after Ach, column C = following intracoronary nitroglycerin. Top panel = treatment with vehicle, mid panel = PTCA only, lower panel = 17 – beta estradiol treatment groups respectively.

Figure 2: Representative light micrographs (x 1000) of cross sections of vessels obtained from the same animal, for immunohistochemical staining with the lectin *Dolichos biflorus* agglutinin (evident as dark brown staining of luminal surface). Vessels treated with 17 – beta estradiol (A) demonstrate reendothelialization to a greater degree as compared to PTCA only (B) and vehicle (C) groups.

Figure 3: Representative light micrographs (x 1000) of cross sections of vessels obtained from the same animal, for immunohistochemical analysis of endothelial nitric oxide synthase (eNOS) expression. Vessels treated with 17 – beta estradiol (A) show greater expression of eNOS (evident as dark brown staining of luminal surface) as compared to PTCA only (B) and vehicle (C) groups.

Figure 4: Graph depicting correlation between vasoconstrictive response to Ach 10^{-4} M and (A) reendothelialization ($r = -0.48$, $p < 0.02$), (B) eNOS expression ($r = -0.58$, $p <$

0.005). Note: % vasoconstriction denotes % decrease in diameter following Ach 10^{-4} M as compared to the basal diameter.

Table: Response to Intracoronary Acetylcholine

Ach*	Diameter-basal (mm)	Diameter-post Ach (mm)	p value
PTCA group			
10^{-7}M	2.79 ± 0.35	2.65 ± 0.35	0.4
10^{-6}M	2.79 ± 0.35	2.54 ± 0.32	0.1
10^{-5}M	2.79 ± 0.35	2.3 ± 0.35	0.02
10^{-4}M	2.79 ± 0.35	1.8 ± 0.48	0.0001
Vehicle group			
10^{-7}M	2.77 ± 0.44	2.6 ± 0.41	0.4
10^{-6}M	2.77 ± 0.44	2.33 ± 0.5	0.06
10^{-5}M	2.77 ± 0.44	2.24 ± 0.47	0.02
10^{-4}M	2.77 ± 0.44	1.89 ± 0.51	0.001
17 – beta estradiol group			
10^{-7}M	2.53 ± 0.6	2.46 ± 0.58	0.8
10^{-6}M	2.53 ± 0.6	2.38 ± 0.58	0.6
10^{-5}M	2.53 ± 0.6	2.36 ± 0.59	0.6
10^{-4}M	2.53 ± 0.6	2.28 ± 0.61	0.4

* acetylcholine

What is claim d is:

1. The use of 17 - beta estradiol in the making of a medical composition or device for preventing restenosis to be administered or installed *in situ* during angioplasty.

5

ABSTRACT

The ndothelium plays a major role in the regulation of structural and functional integrity of coronary arteries. Damage to endothelium occurs following percutaneous transluminal coronary angioplasty (PTCA). Locally delivered 17 - beta estradiol following PTCA in pigs resulted in an enhanced reendothelialization and endothelial function, possibly via a markedly higher endothelial nitric oxide synthase (eNOS) expression when compared to controls. Since endothelial dysfunction can promote both restenosis and coronary spasm, local 17 - beta estradiol delivery is a promising new approach to improve the results after PTCA.